HOST DISCRIMINATION IN *MONODONTOMERUS OBSOLETUS* FABRICIUS (HYMENOPTERA: TORYMIDAE), A PARASITE OF THE ALFALFA LEAFCUTTING BEE *MEGACHILE ROTUNDATA* (FABRICIUS) (HYMENOPTERA: MEGACHILIDAE)

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Abstract.—An experimental investigation of the host discrimination of *Monodontomerus obsoletus,* a gregarious parasitoid of immature alfalfa leafcutting bees, *Megachile rotundata,* was conducted. Naive or experienced parasitoids distinguished two classes of parasitized hosts from unparasitized hosts, based on the time interval since initial parasitization. Hosts parasitized 72 or more hours before exposure to a second female were rarely superparasitized. Hosts parasitized 24 hours prior to exposure to a second female were usually superparasitized but fewer eggs were deposited in second broods than in first broods. A significant inverse correlation was observed between the size of first and second broods in 24-hour hosts. This suggests that superparasitizing females were able to approximate the size of first broods and adjusted the size of their own broods accordingly.

Ovipositing females of many species of parasitic wasps discriminate between unparasitized hosts and those that have been parasitized by conspecifics (Salt, 1961; van Lenteren, 1981). Parasitized hosts may be distinguished by a pheromone applied during parasitization, by tactile or auditory cues (Arthur, 1981; van Lenteren, 1981) or by some form of "internal marker" (Strand, 1986). Responses to some cues may also be modified by experience: in some species, females with oviposition experience are more likely than naive females to reject parasitized cocoons (van Lenteren and Bakker, 1975; van Lenteren, 1976).

This study reports on host discrimination by *Monodontomerus obsoletus* Fabricius, a torymid parasitoid of immature solitary bees. Among its hosts is *Megachile rotundata* (Fabricius), the alfalfa leafcutting bee, the most important commercial pollinator of alfalfa in the Pacific northwest. To better understand the relationship between this parasitoid and its agriculturally important host, experiments were designed to answer the following questions: (1) Can female *M. obsoletus* distinguish parasitized from unparasitized *M. rotundata*? (2) Do female parasitoids distinguish between hosts that differ in the time elapsed since they were parasitized? (3) Are experienced females more likely than naive females to reject parasitized hosts?

The behavior and biology of *M. obsoletus* has been studied in some detail by Eves (1970), and Hobbs and Krunic (1971), who mistakenly referred to it as *M. obscurus* Westwood (E. E. Grissell, pers. comm.). Briefly, females gain entry to a nest of *M. rotundata* where they immobilize immatures by stinging them through the protective leaf sheath and/or cocoon layers. A variable number of eggs is then deposited between the inner cocoon wall and the host [usually 6–10; Eves (1970) reports a range of 3–51 eggs/host but I have rarely found more than 15]. The eggs hatch in about 36 hours

at 29°C, and non-cannibalistic larvae devour the host in 5–6 days. Adults emerge from the host cocoon at about 20 days. The sex ratio of broods is almost invariably biased towards females and sib-mating is probably the predominant breeding system (Hamilton, 1967). In the laboratory, siblings emerge from the cocoon over a short period of time, and mate almost immediately. Indeed, some females mate with their brothers before emerging from the host (Tepedino, 1988a). After mating, the cycle is recommenced either in the same *M. rotundata* burrow, if unparasitized hosts are available, or in a different one. All immature stages of *M. rotundata* are vulnerable to attack although parasitization of early larval instars is rarely successful.

MATERIALS AND METHODS

All *M. obsoletus* used for experiments were randomly selected from individuals emerging from stock cultures maintained at this laboratory. Prepupae of *M. rotundata* were radiographed (Stephen and Undurraga, 1976) in their cocoons and leaf sheaths prior to use so that obviously dead individuals could be detected and eliminated. Healthy-appearing prepupae were used as hosts one day after their removal from winter storage (4–5°C).

Each of 20 naive female parasites was placed in a clean glass petri dish with three host cocoons for 24 hours at 29°C and 16L:8D photoperiod. Each cocoon represented a different treatment: (1) unparasitized; and parasitized by another *M. obsoletus* female (2) 24 hours earlier and (3) 96 hours earlier. Three hosts were chosen because *M. obsoletus* usually parasitizes three or more cocoons in the first 24 hours after emergence. In another study (Tepedino, 1988b), 28 of 29 females presented with six healthy hosts parasitized 3 or more of them in the first 24 hours. Thus females were expected to parasitize all three hosts offered unless one or more of the treatments were unacceptable. All previously parasitized cocoons were kept at 29°C and 16L: 8D from initial parasitization until presentation to naive females. After 24 hours, females were removed, host cocoons were opened and their contents examined. The presence of both eggs and larvae in a cocoon indicated superparasitism. Because eggs hatch in about 36 hours (Eves, 1970), only those from the first brood in treatments 2 and 3 would have hatched into larvae.

A second group of 10 freshly emerged, naive females were each presented with one healthy, unparasitized cocoon in a glass culture dish at 29°C and 16L:8D photoperiod for 24 hours. The cocoons were then removed, and the wasps held at 25°C in the dark for 72 hours. During this time females were fed a drop of 50% honey solution applied to an unused cigarette filter inserted into a sidearm in the dish. At the end of the 72 hours, females were presented with three experimental cocoons as described above for naive females except that the 96 hour treatment was replaced by a cocoon which had been parasitized 72 hours before. Subsequent treatment was as described above for naive females.

RESULTS

Naive female *M. obsoletus* parasitized 34 of 57 available hosts. [Each of three replicates contained a dead host undetected by radiography. All three were victims of the fungal disease, chalkbrood, *Ascosphaera* (McManus and Youssef, 1984)]. No female parasitized all three hosts; fourteen females attacked two hosts each; six

Table 1. Mean number of eggs deposited by naive females in unparasitized or parasitized cocoons and the mean number of larvae in the pre-parasitized cocoons. N_L = total number of cocoons with larvae available; N_E = total number of cocoons with eggs.

Cocoon treatment	Mean number (+SD)			
	N_E	Eggs	N_L	Larvae
Unparasitized	18	9.2 (4.8)	—	—
Parasitized 24 hrs	13	5.8 (4.1)	18	8.2 (2.4)
Parasitized 96 hrs	3	5.0 (3.0)	19	10.4 (1.9)

females attacked one host each. As in a previous study (Tepedino, unpublished), females that parasitized two hosts deposited fewer eggs per host (6.9 ± 1.7) than those that parasitized only one host (10.2 ± 4.6) ($t = 2.39$, $P < 0.05$).

Naive females clearly avoided hosts parasitized by conspecifics 96 hours earlier. Of the hosts available (20 unparasitized, 18 parasitized 24 hours earlier, 19 parasitized 96 hours earlier), significantly fewer of those parasitized 96 hours earlier were attacked than those of the other two categories (Table 1, $\chi^2 = 22.8$, df = 1, $P < 0.001$). There was no significant difference in frequency of attack between unparasitized cocoons and those parasitized 24 hours earlier ($\chi^2 = 1.2$, df = 1, $P > 0.25$).

Important differences were detected among host treatments in the number of eggs deposited per host. Although the frequency of attack upon unparasitized hosts and those parasitized 24 hours earlier did not differ, there was a significant difference between them in the number of eggs laid per host (Table 1). Naive females deposited significantly more eggs in unparasitized hosts than in those parasitized 24 hours earlier ($t = 2.02$, df = 28, $P = 0.05$).

Indirect evidence suggests that naive females in the choice experiments parasitized unparasitized hosts before superparasitizing 24- and 96-hour hosts. Each 24- and 96-hour host received its first brood from a single naive female. The number of eggs laid by these females (Table 1, Larvae) thus represent a useful standard against which to compare the number of eggs deposited by naive females in the choice experiments. There was no difference in the size of first broods in 24-hour hosts, 96-hour hosts or unparasitized hosts in the choice experiments (ANOVA, F = 2.26, df = 2,51, $P > 0.10$). This finding, coupled with the significant differences among treatment types reported above, suggests that females attacked unparasitized hosts first, possibly after investigating other available hosts. The data also suggest that superparasitizing females adjusted the number of eggs deposited to the number of eggs already present in the host. There was an inverse relationship between the number of eggs laid by first and second females on 24-hour hosts (Fig. 1), but it was not significant ($r = -0.47$, df = 12, $P = 0.10$).

The behavior of females with a single previous oviposition experience closely resembled that of inexperienced females. Although sample sizes were diminished by the presence of ten moribund but undetected victims of chalkbrood, results were unambiguous: first, all seven unparasitized hosts were parasitized, all six 24-hour hosts were superparasitized, but none of the seven 72-hour hosts were superparasitized. Second, like their naive counterparts, experienced females laid fewer eggs in hosts parasitized 24 hours earlier than in unparasitized hosts (8.2 ± 4.4 vs. 10.3 ±
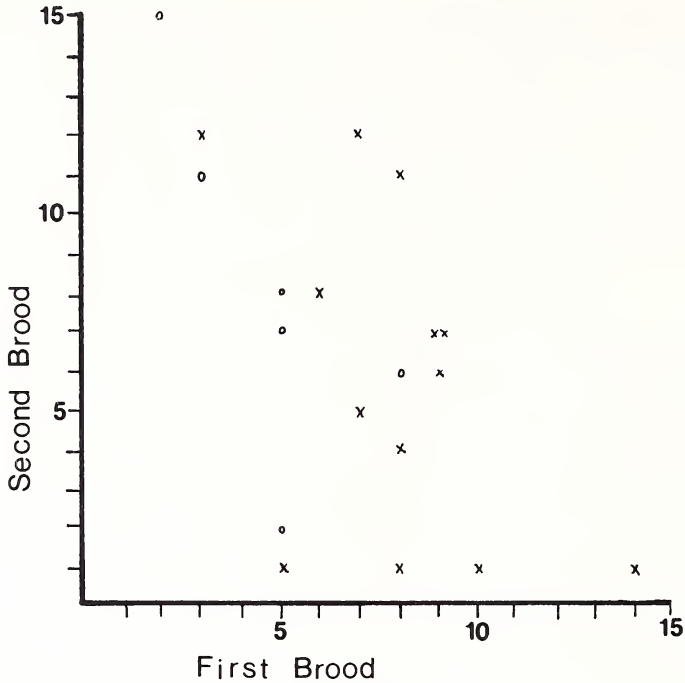
Fig. 1.   The number of progeny from first and second (superparasitism) broods of *M. obscurus* in prepupae of *M. rotundata* (Y = −0.85x + 12.45).

2.3) but in this case the result was not significant (t = 1.11, df = 5,6, P > 0.25). And third, an inverse, but non-significant, correlation was found between the number of eggs deposited by experienced females in 24-hour cocoons and the number of eggs already present in the cocoon (Fig. 1; r = −0.71, df = 5, P = 0.11). If the results for superparasitism of 24-hour hosts are combined for experienced and naive females, as is permissible because they did not differ in oviposition behavior, then the inverse correlation between the number of eggs laid in first and second broods is significant (Fig. 1; t = 2.87, df = 17, P = 0.01).

DISCUSSION

Monodontomerus obsoletus may be added to the burgeoning list of hymenopterous parasitoids that distinguish unparasitized hosts from those parasitized by conspecifics. Unlike some other species (van Lenteren and Bakker, 1975; van Lenteren, 1976), discriminatory ability of females was not improved by prior oviposition experience with an unparasitized host.

In general, the results for *M. obsoletus* resemble those for *Nasonia vitripennis* (Walker) (Werren, 1984). In both studies essentially four classes of prospective hosts were distinguished by ovipositing females: (1) unparasitized-acceptable; (2) parasitized-acceptable; (3) parasitized-unacceptable; and (4) unparasitized-unacceptable

(moribund). The obvious difference between hosts of classes 2 and 3 is the time since initial parasitization. For both species a positive relationship was found between percent hosts rejected and the time since the host was first parasitized. Other examples of similar behavior are cited by Strand (1986). In this study, hosts parasitized at least 72 hours before being offered to naive females were superparasitized only rarely, but those parasitized 24 hours earlier were superparasitized almost without exception. Distinctions between host classes may be made by whether feeding larvae are present in the host cocoon or not [in *M. obsoletus* eggs hatch at about 36 hours and first instar larvae begin to feed as soon as they attach to the host (Eves, 1970)], or by the presence of external or internal markers, or a combination of these (Werren, 1984; Strand, 1986). Studies are underway to address some of these questions.

Until recently, the conception of superparasitism as a cost of either inexperience or error was based on the surmise that progeny of superparasitizing females were invariably doomed (Salt, 1961; van Lenteren, 1981). Under such circumstances females would be under strong selective pressure to distinguish unparasitized hosts from those parasitized by conspecifics and to avoid the latter. However, several researchers (van Alphen and Nell, 1982; Charnov and Skinner, 1984; Parker and Courtney, 1984; Werren, 1984; Bakker et al., 1985; Skinner, 1985; Waage, 1986) have recently pointed out that superparasitism is to be expected in some situations. For example, superparasitism is likely to be advantageous when the probability of finding unparasitized hosts is low and there is a positive probability that some progeny of the second brood will survive, particularly if egg production is not a concern. Recently, Bakker et al. (1985) have provided empirical support for this prediction by showing that some progeny of superparasitizing females can survive, at least when inter-oviposition time is short (1 to 3 hours).

Werren (1984) drew attention to two other attributes that would improve the fitness of superparasitizing females. The chances of survival of progeny from second broods would be increased if females could approximate the size of first broods and adjust their own brood size in a compensatory manner. Those females could increase their fitness further by increasing the fraction of males in second broods because of the bias toward females in first broods. Both *M. obsoletus* (Fig. 1) and *N. vitripennis* (Werren, 1984) appear, in an approximate way, to adjust the size of secondary broods. *Nasonia vitripennis* females also appear to adjust the sex ratio of second broods as predicted by the theory (Werren, 1984; but see Orzack and Parker, 1986). Possible changes in the sex allocation pattern of superparasitizing *M. obsoletus* females are being investigated.

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